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Guillaume Vogin, Thierry Bastogne, Larry Bodgi, Julien Gillet-Daubin, Aurélien Canet, et al.. The pATM Immunofluorescence assay: a high-performance radiosensitivity assay to predict post radiotherapy overreactions.. International Journal of Radiation Oncology, Biology, Physics, 2018, 101 (3), pp.690-693. 10.1016/j.ijrobp.2018.03.047 . hal-01757771

HAL Id: hal-01757771

<https://hal.science/hal-01757771>

Submitted on 3 Apr 2018

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The pATM Immunofluorescence assay: a high-performance radiosensitivity assay to predict post radiotherapy overreactions

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Running title:

Updated performance of the pATM radiosensitivity assay

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Conflicts of interest

GV and AC have nothing to disclose.

TB is Co-Founder, Scientific Expert in Biostatistics and Nano-informatics in CYBERNANO company (Biosignal Processing & Biostatistics).

SP and LB report the following patents: FR3040178A1 FR3040179A1 WO2017098190A1 WO2017029451A1 WO2017029449A1 licensed to NEOLYS Diagnostics. SP is Head of R&D at NEOLYS Diagnostics. JGD is co-founder and CEO of NEOLYS Diagnostics.

NF reports the following patents: FR3040178A1, FR3040179A1, FR3017624A1, FR3017625A1, WO2015121597A1, WO2017098190A1, WO2017029451A1, WO2015121596A1, WO2017029449A1. NF is co-founder and Scientific Advisor of NEOLYS DIAGNOSTICS.

Authorship

GV drafted the manuscript and helped in the acquisition of data. AC and LB performed the primary statistical analyses. TB performed the secondary analyses. NF, JGD and SP interpreted the data. All authors revised the manuscript critically and have approved the final article.

Funding

This work was supported by NEOLYS Diagnostics.

The pATM Immunofluorescence assay: a high-performance radiosensitivity assay to predict post radiotherapy overreactions

Running title: Updated performance of the pATM radiosensitivity assay

SUMMARY

A new statistical analysis was performed on 117 patient-specific fibroblasts lines cultured from a skin biopsy in order to assess AUC, sensitivity, specificity, the positive predicted value and the negative predicted value of the pATM Immunofluorescence assay to sort overreactors. The pATM assay shows the highest predictive performance amongst the available tests.

ABSTRACT

Purpose

Identifying prior treatment, the patients who will overreact to radiotherapy (RT) would have sound positive clinical implications. By focusing on DNA double-strand breaks (DSB) recognition and repair proteins after irradiation, we recently demonstrated that the maximal number of pATM nuclear foci in the first hour (pATMmax) after ex vivo irradiation correlated with post-RT toxicity severity. We aimed to carry out additional analyses on our whole collection of fibroblast lines to refine the predictive performance of our assay.

Material and Methods

Immunofluorescence experiments were performed on 117 primary skin fibroblast lines irradiated at 2 Gy. The toxicity response was split up into two binary classes: 0 if toxicity grade < 2 and 1 otherwise. To assess the relationship between the quantity of pATMmax foci and toxicity grade, we applied a correlation then a supervised classification analysis. Training datasets from 13 radiosensitive patients randomly drawn with a random under-sampling technique were constituted. ROC analyses were performed with a Monte-Carlo method to estimate the optimal threshold and discriminate the responses for each data set. The discrimination cut-off was estimated as the maximum value of the 10^4 thresholds computed from each training subset.

Results

As expected, we confirmed a quasi linear dependence between toxicity and pATMmax (Fig 1A & 1B; Pearson correlation coefficient: -0.85; $p < 2.2 \times 10^{-16}$). When taken as a binary predictive assay with the optimal cut-off value of 34.5 pATM foci/cell, our assay showed an outstanding predictive performance with the following values respectively for sensitivity, specificity, NPV, PPV and AUC: 1.00, 0.92, 1.00, 0.99 and 0.987.

Conclusion

The results of these experiments allowed us to identify pATMmax as a high performance predictive parameter of post-radiotherapy OR. Additional studies are in progress to confirm that this radiosensitivity assay reaches the same performances in any condition to adapt the clinical practice.

Introduction

Up to 15% of the patients treated with radiotherapy (RT) experience toxicity that can lead to serious sequelae and additional societal costs (1-3). In particular, their cumulative incidence at least 30 years after the diagnosis of cancer reaches 73.4% in pediatric oncology (4). Identifying these over reactors (OR) prior RT would therefore have sound positive clinical implications.

The severity of toxicity depends on several factors dominated by individual radiosensitivity (iRS) (5). iRS could be defined as the innate capacity of a given individual to show a particular toxicity profile to radiation - corresponding to the loss of proliferative capacity observed in vitro.

Since 2003, we have collected primary skin fibroblasts from patients with DNA repair deficiencies, from apparently healthy patients or from irradiated patients with various severity grades and free intervals after RT in more than 30 French or Belgian hospitals. According to Baumann et al., OR are unselected patients who experienced severe side effects defined as follows, after the same standardized physical radiation dose, without evidence of treatment- or patient-related cofactors that can affect radiation tolerance (6) :

- grade ≥ 2 occurring the first two weeks of RT where these side effects are unexpected or
- grade ≥ 3 lasting more than 4 weeks after the end of RT or
- grade ≥ 3 occurring or persisting more than 90 days after the end of RT (7).

We measured the RT-induced distribution of candidate DNA double-strand breaks (DSB) recognition and repair proteins with an immunofluorescence assay (8). We thus recently proposed a general classification of Human iRS based on the nucleoshuttling of ATM kinase (9). However, we described a continuous distribution of the maximal number of pATM nuclear foci in the first hour (pATMmax) after ex vivo irradiation according to the 6 possible severity grades taken individually – that is not suitable to support clinical decision making (9). During ESTRO36, we reported a preliminary ROC curve analysis according to a binarized toxicity grade classification. With the optimal cut-off value of 35 foci, pATMmax assay showed promising predictive performances, with an area under the curve (AUC) of 0.97, a predictive positive value (PPV) of 99%, a specificity of 92% and a sensitivity of 100% (10).

Our objective in the present letter was to refine the performance of our iRS assay on the whole cohort applying a new analysis with a robust supervised classification.

Material and Methods

Material description

This study was conducted on the 117 primary skin-untransformed fibroblast lines we previously reported including 12 radioresistant, 4 ATM^{-/-}, and 1 LIG4^{-/-} gifted cell lines and 100 lines from OR patients (9). Among the irradiated patients in our cohort, the median time from treatment to biopsy was 13.3 months (1-273). Two independent expert radiation oncologists graded the toxicity severity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The toxicity response was split up into two binary classes: 0 if toxicity grade < 2 ("biologically radioresistant") and 1 otherwise ("biologically radiosensitive")

The regional ethical committee approved the COPENIC collection. Cell lines were declared under the numbers DC2008-585 and DC2011-1437 to the French Ministry of Research. The database was protected under the reference as IDDN.FR.001.510017.000.D.P.2014.000.10300. All the patients were informed and gave signed consent according to the ethics recommendations.

The biological methods have been extensively described (8, 11).

Statistical analysis

Assessment of quality. To assess the relationship between the quantity of pATMmax foci – defining iRS - and binarized toxicity grade, we applied a Pearson correlation test. The intra-grade variance is defined as the variance of pATMmax foci in each CTCAE group. A Student t-test and a Fisher test were carried out to examine the relevance of the affine model parameters and the adequacy between the model response and the experimental data respectively. In each test, the associated p-value indicates the compatibility of data with the null hypothesis.

Supervised classification. Our initial dataset was expected unbalanced between "radiosensitive" and "radioresistant" patients. To correct it, a random under-sampling technique was implemented, in which 13 radiosensitive patients were randomly drawn from the 104 available subjects (12). As a consequence, each training dataset was first composed of 26 patients (13 radiosensitive and 13 radioresistant patients). The thirteen

radioresistant subjects were always the same in this study. ROC analyses were performed with a Monte-Carlo method to estimate the optimal threshold and discriminate the responses for each data set. Finally, the discrimination cut-off was estimated as the median value of the 10^4 thresholds computed from each training subset. The classification performances were characterized on the 117 patients by five parameters: AUC, sensitivity index, specificity index, the PPV and the negative predicted value (NPV). The analyses were carried out on R.

Results

Two independent expert radiation oncologists defined the severity grade in blind with total agreement.

Bivariate Analysis

As expected, we clearly emphasized a quasi-negative linear dependence between toxicity and pATMmax (Fig 1A; Pearson correlation coefficient: -0.85). The t-test and F-test confirmed the strong correlation between those two quantities reflecting the degradation of pATM nucleoshuttling with worsening of toxicity (p-values $< 2.2e^{-16}$ for the two tests).

Supervised classification analysis

We reclassified 104 radiosensitive patients and 13 radioresistant subjects. After the Monte-Carlo session, the final classification cut-off was estimated at 34.5 pATMmax foci/cell nucleus. From the ROC curve, we obtained the following values respectively for the sensitivity, specificity, NPV, PPV and AUC: 1.00, 0.92, 1.00, 0.99 and 0.987 (Fig 1B). We estimated the coefficients of variation of each quality criterion in the same order: 0, 0.019448, 0.023315, 0 and 0.0076695.

Discussion

To our knowledge, our pATMmax predictive assay performed on skin fibroblasts show the highest performance reported so far – pointing ATM nucleoshuttling as a very good predictor for Human iRS (13-15). This quantitative correlation was found to be independent of tumor location, cell type and of early or late occurrence of the toxicity (9). Unlike other assays reporting molecular or cellular endpoints like polymorphisms or apoptosis, our assay provides: 1) a reliable prediction of radiosensitivity by considering CTCAE grades separately – and in a binarized way (9); 2) a relevant biological interpretation of the linear-quadratic model (16-18); 3) a relevant mechanistic

model to explain major IRS in genetic diseases associated with mutations of cytoplasm proteins interacting with pATM nucleoshuttling (11, 19, 20). Patients are currently enrolled prior RT in confirmatory prospective studies in pediatrics (NCT02827552) and sarcomas/GI malignancies (NCT02797405).

We are aware that the mandatory cellular amplification step after skin sampling requires several weeks that could appear not suitable for clinical use. However, we are now developing a fast ELISA-assay targeting pATM directly from the original skin sample and with still very interesting performances (average AUC of more than 0.8) and a deadline of a few days for editing the results (14).

Conclusion

When taken as a binary predictive assay, the pATMmax predictive assay appears as one of the most powerful available. Prospective studies are in progress to confirm its performance.

FIGURE CAPTION

A. Correlation analysis between the mean number of pATM foci/cell nucleus and the individual CTCAE grades. Each cross denotes a radioresistant patient and each circle a radiosensitive subject. The red line represents the regression providing the best fit to experimental data. B. ROC curve computed from the whole reclassified data set, AUC=0.987 (CV=0.0076695)

REFERENCES

1. UNSCEAR. UNSCEAR 2008 REPORT Vol. II : EFFECTS OF IONIZING RADIATION. Publications UN, editor. Vienna: United Nations; 2008. 320 p.
2. Mohanti BK, Bansal M. Late sequelae of radiotherapy in adults. Support Care Cancer. 2005;13(10):775-80.
3. Rapport 2013 de l'Observatoire sociétal des cancers. 2014.
4. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, et al. Chronic health conditions in adult survivors of childhood cancer. The New England journal of medicine. 2006;355(15):1572-82.

5. Safwat A, Bentzen SM, Turesson I, Hendry JH. Deterministic rather than stochastic factors explain most of the variation in the expression of skin telangiectasia after radiotherapy. *International journal of radiation oncology, biology, physics*. 2002;52(1):198-204.
6. Bentzen SM, Overgaard J. Patient-to-Patient Variability in the Expression of Radiation-Induced Normal Tissue Injury. *Semin Radiat Oncol*. 1994;4(2):68-80.
7. Baumann M, Holscher T, Begg AC. Towards genetic prediction of radiation responses: ESTRO's GENEPI project. *Radiother Oncol*. 2003;69(2):121-5.
8. Joubert A, Zimmerman KM, Bencokova Z, Gastaldo J, Chavaudra N, Favaudon V, et al. DNA double-strand break repair defects in syndromes associated with acute radiation response: at least two different assays to predict intrinsic radiosensitivity? *International journal of radiation biology*. 2008;84(2):107-25.
9. Granzotto A, Benadjaoud MA, Vogin G, Devic C, Ferlazzo ML, Bodgi L, et al. Influence of Nucleoshuttling of the ATM Protein in the Healthy Tissues Response to Radiation Therapy: Toward a Molecular Classification of Human Radiosensitivity. *International journal of radiation oncology, biology, physics*. 2016;94(3):450-60.
10. Vogin G, Bodgi L, Canet A, Pereira S, Gillet-Daubin J, Foray N, editors. High-performance radiosensitivity assay to predict post radiation overreactions. *ESTRO 36*; 2017; Vienna, Austria.
11. Ferlazzo ML, Sonzogni L, Granzotto A, Bodgi L, Lartin O, Devic C, et al. Mutations of the Huntington's disease protein impact on the ATM-dependent signaling and repair pathways of the radiation-induced DNA double-strand breaks: corrective effect of statins and bisphosphonates. *Molecular neurobiology*. 2014;49(3):1200-11.
12. Ganganwar V. An overview of classification algorithms for imbalanced datasets. *International Journal of Emerging Technology and Advanced Engineering*. 2012;2(4):42-7.
13. Ferlazzo ML, Bourguignon M, Foray N. Functional Assays for Individual Radiosensitivity: A Critical Review. *Semin Radiat Oncol*. 2017;27(4):310-5.
14. Pereira S, Bodgi L, Duclos M, Canet A, Ferlazzo ML, Devic C, et al. Fast and binary assay for predicting radiosensitivity based on the nucleoshuttling of ATM protein: development, validation and performances. *International Journal of Radiation Oncology • Biology • Physics*. 2018;100(2):353-60.
15. Pereira S, Bodgi L, Duclos M, Canet A, Ferlazzo ML, Devic C, et al. In reply to Azria D ; The time is coming to compare radiosensitivity predictive assays by their scientific relevance and statistical performances. *International Journal of Radiation Oncology • Biology • Physics*. 2018;In Press.
16. Bodgi L, Canet A, Pujo-Menjouet L, Lesne A, Victor JM, Foray N. Mathematical models of radiation action on living cells: From the target theory to the modern approaches. A historical and critical review. *Journal of theoretical biology*. 2016;394:93-101.
17. Bodgi L, Foray N. The nucleo-shuttling of the ATM protein as a basis for a novel theory of radiation response: resolution of the linear-quadratic model. *Int J Radiat Biol*. 2016;92(3):117-31.
18. Bodgi L, Granzotto A, Devic C, Vogin G, Lesne A, Bottollier-Depois JF, et al. A single formula to describe radiation-induced protein relocalization: towards a mathematical definition of individual radiosensitivity. *Journal of theoretical biology*. 2013;333:135-45.
19. Ferlazzo ML, Foray N. Huntington Disease: A Disease of DNA Methylation or DNA Breaks? *Am J Pathol*. 2016;186(7):1750-3.
20. Ferlazzo ML, Bach-Tobdji MKE, Djerad A, Sonzogni L, Devic C, Granzotto A, et al. Radiobiological Characterization of Tuberous Sclerosis: a Delay in the Nucleo-Shuttling of ATM May Be Responsible for Radiosensitivity. *Molecular neurobiology*. 2017.